

Age Factors Potentiating Drug Toxicity in the Reproductive Axis

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Traditionally, drug toxicity in the reproductive system has been a concern only as it affects fertility and fecundity in young individuals. The purpose of this report is to address the potential problem of synergy between drug actions and abnormal secretion of reproductive hormones that together produce disease in older individuals. Thus, reproductive toxicity has different, but no less serious implications in aging individuals. During aging, the coordinated function of elements within the reproductive neuroendocrine axis degrades. This change promotes atypical secretion of hormones producing abnormal responses in target organs and thus creates a condition with pathogenic potential. Certain drugs may contribute to reproductive toxicity in aging individuals either by accelerating the process of dysregulation and/or by synergizing with hormones to stimulate pathologic changes in target tissues. The geriatric population of the world is increasing, and since it consumes a proportionately larger percentage of drugs than younger groups, this novel form of reproductive toxicity may represent a problem in drug safety that warrants serious consideration.

Introduction

Postmenopausal women sometimes participate in phase I clinical trials of drug development because they lack menstrual cycles, a fact that seemingly precludes the need for concern about potential reproductive toxicity. The disregard for reproductive consequences of drug treatment in these subjects seems perfectly appropriate, since intuitively, the issue should be relevant only to those women with ovarian cycles who are capable of becoming pregnant and bearing offspring. However, more in-depth consideration reveals that this attitude focuses concern for reproductive toxicity only indirectly toward the individual being medicated. In effect, the prime focus of reproductive toxicity studies is, in general, the future generation, with minimal regard for the present one except to ensure that the drug(s) in question does not alter its capacity to reproduce. That the goal of reproductive toxicity determinations is ultimately to avoid drug-induced reproductive dysfunction is obvious, and to criticize this goal is not the intent of the present argument.

Instead, it is to suggest that an equally valid goal of reproductive toxicity studies is to define interactions of drugs with components of the hypothalamo-pituitary-ovarian axis that may be detrimental to the health of the treated individual. For example, since cyclic ovarian function is absent in postmenopausal women, it would seem that the question of whether or not a drug per-

turbs ovarian cycles in these individuals is moot. However, despite the fact that ovarian activity in aged women is not cyclic, elements of the reproductive axis continue to function—albeit abnormally—creating a hormonal milieu that can be pathogenic. Thus, abnormal reproductive function in peri- and postmenopausal women may increase their risk for developing certain types of disease. Compounding this problem is the potential for toxic interactions of drugs with reproductive hormones whose secretion has become abnormal. Synergistic effects of these factors may have significant ramifications upon the patient's health. An example of this toxic potential that has received notoriety over the past few years is the increased incidence of uterine and breast cancer resulting from treatment of postmenopausal women with estrogenic compounds (1-4). Attempts to use these compounds to reverse or retard degenerative diseases derive from the fact that the diseases result—at least in part—from declining ovarian function. To a certain degree, replacement therapy has been successful. However, treatment has also increased the incidence of certain neoplastic diseases, notably those of the reproductive organs.

As discussed below, these findings demonstrate that reproductive aging does not result from simple loss of estrogen production. Instead, the ovary degenerates, and normal patterns of estrogen production and secretion fall as a consequence of age changes that undermine integrated activity within the entire reproductive neuroendocrine axis. The impact of these changes upon the organism is much more significant than simple depletion of ovarian primordial follicles. Loss of integrated activity leads to internal chaos in a system that requires

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strictly ordered events for proper function and maintenance of health. In this fragile state, the menopausal female is more apt to suffer toxic effects from drugs that produce only modest responses in younger individuals. Thus, the purpose of this paper is to analyze changes that occur in reproductive function during senescence, focussing upon the possible ramifications of such change in terms of enhanced drug toxicity and resultant pathology in the aging female.

Changes in Reproductive Function during Aging

The major hypothesis of this study is that the potential for drug toxicity is amplified in the dysregulated reproductive neuroendocrine axis of aging females. Thus, a brief description of hormonal changes that occur in females during senescence is an appropriate prelude to analysis of putative mechanisms for such toxicity.

Figure 1 shows typical levels of reproductive hormones in reproductive and postreproductive women (5). Since gonadotropin levels are high and steroids are low in the older group, the data suggest that during aging the ability of the ovary to produce estrogen declines. This in turn leads to compensatory output of pituitary gonadotropins. Support for this view derives from the histology of ovaries from postmenopausal women that clearly lack primordial follicles (6). Thus, it has been suggested that simple depletion of these germinal centers accounts for reproductive senescence in women (7). However, more careful scrutiny of clinical reports showing hormonal profiles of women in transition from cyclic to acyclic states, as well as analysis of data from animal studies, suggests that age changes in female reproductive function occur initially at extraovarian sites, then

spread throughout the axis to finally impact upon the ovary. As discussed below, the subtle and progressive alterations in reproductive homeostasis have potentially greater health implications than simple decline in estrogen production by the ovary.

As female rodents (8) and primates (9) approach middle age and beyond, their reproductive cycles increase in length as the frequency of ovulation decreases. This effect is due in part to alterations in the pattern(s) of gonadotropin secretion. In rats, progressive delay in onset and attenuation of the preovulatory surge of luteinizing hormone (LH surge) continues until the temporal and quantitative perturbations cause ovulatory irregularity. These changes are summarized in Figure 2. Presumably, altered gonadotropin release results ultimately from CNS perturbations affecting at least the metabolism and secretion of monoamines (10-12). In general terms, these changes are reciprocal between catecholamines and serotonin (13), ultimately shifting the nature of monoamine signals to the pituitary from catecholamines to those predominated by serotonin. This imbalance of monoamine signals leads not only to a decremental gonadotropin secretion, but also to hypersecretion of prolactin (14), a condition which has significant health implications and is directly related to the problem of drug toxicity and reproductive senescence.

Paradoxically, a concomitant of these changes at the ovarian level is different from what is expected when examining end-stage ovaries from postmenopausal females. During cycle extension at the perimenopausal or periostropausal interval, quite often estrogen release from the ovary is enhanced rather than retarded. As seen in Figure 3, the enhancement is not reflected by increased peak levels of the steroid in serum, but rather by extended release at relatively high levels. This altered secretion of estrogen may result from the fact

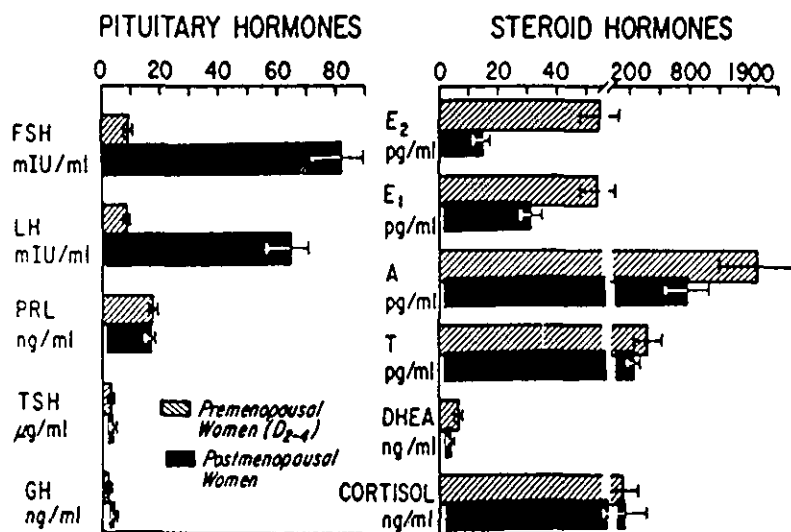


FIGURE 1. Circulating levels of pituitary and steroid hormones in postmenopausal women as compared with premenopausal women studied during the first week (days 2 to 4) of the menstrual cycle. From Yen (5).

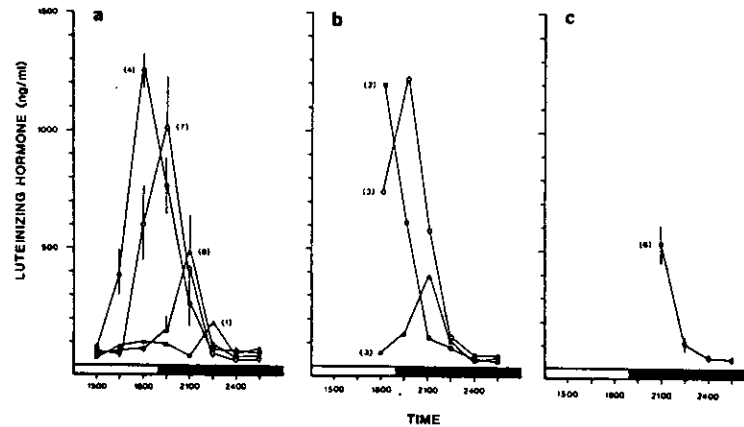


FIGURE 2. Serum LH (mean \pm SEM) in middle-aged females on the afternoon and evening of vaginal proestrus. Sampling was initiated (a) at 1500 hr, (b) 1800 hr and (c) 2100 hr. Animals were grouped according to the time their LH peak was observed. In (a) the maximum LH value for the rats in which the LH peak was observed at 2100 hr (Δ) was significantly lower than the maximum LH values observed in those rats which peaked at 1800 hr (\circ) and 1930 hr (\bullet) (Student's *t* test, $p < 0.05$). The maximum LH values of those rats in which the LH peak was observed at 1800 hr and 1930 hr were not significantly different than the peak LH values observed in young rats. The number of animals in each group is indicated in parentheses. The SEM is indicated only for those groups composed of four or more rats. From Cooper et al. (11).

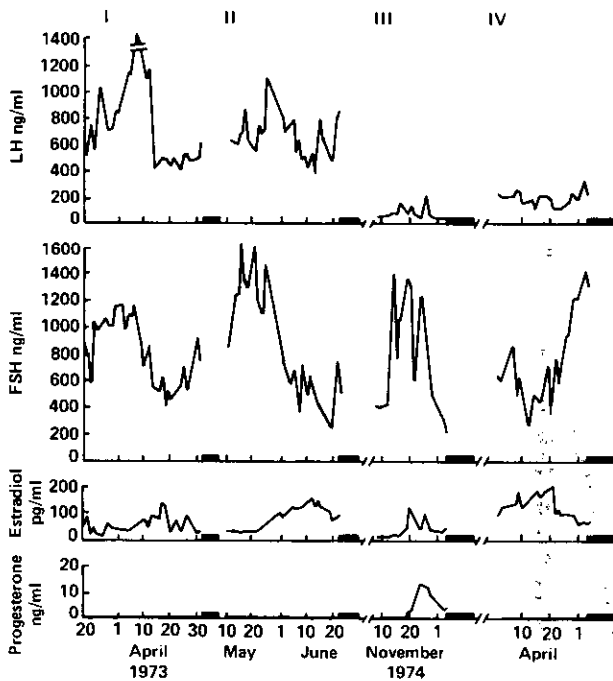


FIGURE 3. Daily concentrations of serum LH, FSH, E_2 , and P during four cycles in one 49-year-old subject during the menopausal transition. Hormone levels are arrayed by calendar date and the hatched area indicates menstruation. From Sherman et al. (9).

that at the perimenopause, graafian follicles tend not to ovulate, but instead they accumulate in the ovary and sometimes become cystic (7,15,16).

Similar changes that occur in the rodent ovary as the animal enters constant estrus (8) are apparently responsible for changes in secretory patterns and serum levels of estradiol. Estrogen depresses LH secretion

and stimulates prolactin release from the pituitary (17). Thus, these ovarian changes resulting from the initial perturbation exacerbate and accelerate dysregulation within the reproductive axis. A positive feedback relationship develops which ultimately becomes catastrophic to cyclic reproductive function. This catastrophic cascade phenomenon can be demonstrated experimentally when gonadotropins are depleted with antibodies. The depletion produces anticipated drops in gonadal steroidogenic capacity and is also associated with enhanced prolactin secretion. However, when the primary perturbation (gonadotropin depletion) is maintained, irreversible secondary damage ultimately occurs to germinal elements, and the functional capacity of the gonad is irrevocably lost (18,19). Thus, experimental suppression of gonadotropin is sufficient to cause permanent damage to the gonad. Examination of the product of this experimental manipulation reveals an animal with atrophic gonads and a hypergonadotrophic pituitary. The real significance of the model would be missed if the transition state, when gonadotropins were low and prolactin was high, were ignored. During the transition, conditions occur which clearly allow toxicity of certain drugs to be expressed. In the natural condition (during aging), gonadotropins are not blocked by antibodies. However, erosion of neuroendocrine mechanisms that time critical events within the reproductive axis, such as preovulatory release of gonadotropin, have the same effect. Rather than eliminating gonadotropins, the temporal presentation of gonadotropins to the ovary is improper, producing inappropriate responses from an ovary that is physiologically unprepared for its ovulatory stimulus.

Thus, the seemingly innocuous temporal changes in gonadotropin secretion during aging have serious functional implications because they undermine the time re-

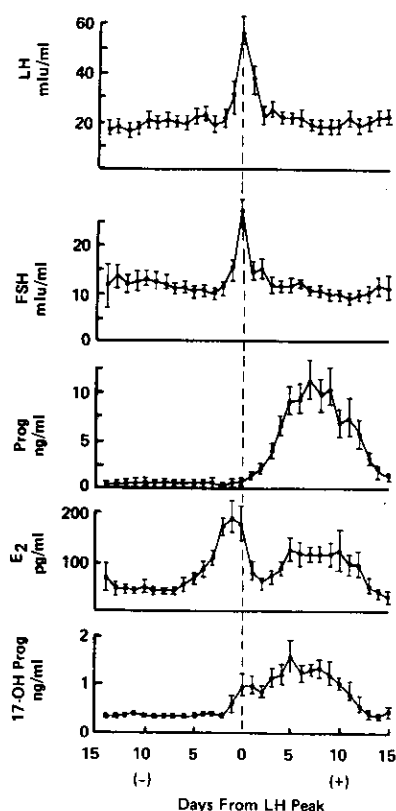


FIGURE 4. Mean LH, FSH, progesterone, estradiol, and 17-OH progesterone levels in blood specimens collected daily throughout presumptive ovulatory menstrual cycles from normal women. Cycle days were synchronized around day of LH peak = day 0. From Thorneycroft et al. (39).

relationships that are elemental to functional integration of the total axis. In addition, poorly regulated release of reproductive hormones from the pituitary and inappropriately stimulated ovaries, in turn, cause target tissues to receive abnormal stimulation. This situation has the potential to produce aberrant responses in the target cells, increasing in some cases, their risk of developing disease.

Estrogen and Prolactin

Although estrogen and prolactin perform functions that are essential for reproductive cycles in females, they also are potent substances having the potential to cause degenerative change and disease. As seen in Figure 4, the rise and fall of estrogen and prolactin in conjunction with other endocrine factors are temporally correlated with events needed for ovulation. Of significance is the fact that the negative effects of estrogen and prolactin are expressed when the limits of exposure defined by the temporal constraints of normal reproductive cycles are violated. Thus, in the aging female, cycles are irregular, and hormone secretions are not coordinated, providing an environment in which their pathogenic potential is realized. During cycle dysreg-

ulation in the middle-age transition, estrogen release is often prolonged (Fig. 3).

The significance of this phenomenon is demonstrated in animal studies that test the effect of estrogen on cell viability in hypothalamic loci that regulate reproductive cycles (20). The studies show that prolonged administration of estrogen causes cycle instability and ultimately cycle failure (20,21). These effects are presumably mediated by an impact of the steroid on the brain, where altered metabolism of hypothalamic monoamines and degeneration of neurons in the arcuate nucleus follow administration of estrogen (21,22). Pharmacodynamic changes include alterations in the metabolism of serotonin (22), whose intermittent metabolism and secretion has been linked to phasic secretion of LH (23,24). Estrogen abolishes the rhythmic metabolism of brain serotonin, thus perturbing the signal needed for phasic release of LH. Furthermore, estrogen also stimulates prolactin release from the pituitary. Since as previously described, prolactin hypersecretion causes degeneration of germinal centers within the gonads (25) and also promotes the growth of mammary and pituitary tumors (26,27), its release increases the incidence of pathology.

The relevance of these facts to discussion of the reproductive system and drug toxicity derives from the fact that the aged are often treated with drugs that have the potential to accelerate the changes in estrogen and/or prolactin secretion that normally occur during aging. These effects increase the chances for realizing the pathogenic potential of the drugs. For example, aging women suffer symptoms of the menopause that can be related to estrogen deficits. Based upon the understanding of reduced steroidogenic capacity of the aging ovary shown in Figure 1, estrogenic compounds have been administered in an attempt to relieve these symptoms. However, as the results of such "replacement therapy," these women increase their risk of developing diseases of the reproductive tract and mammary glands. Thus, while reasonable in concept, replacing a deficient hormone does not only accomplish the desired effect, it increases the risk of developing disease by exacerbating the process of senescence in the reproductive system itself.

In addition to these estrogenic effects on the reproductive tract, the incidence of neoplastic disease affecting the mammary gland and pituitary also increase with aging. Certain drugs synergizing with these changes can thus show increased reproductive toxicity. These can occur in estrogenic compounds as well as in certain neuroleptic compounds that alter monoamine metabolism.

Serotonergic Drugs and Pituitary Neoplasms

Pituitary adenomas occur more frequently in aged than in young animals, with females having the higher incidence (27-29). Although the reasons for the sex difference not established conclusively, it probably results

Table 1. Anterior pituitary 5-HT content in rats of different age and reproductive status.^a

| Group | N | Anterior pituitary weight (mg \pm SEM) | Anterior pituitary 5-HT, pg \pm SEM | |
|-------------|---|--|---------------------------------------|----------------|
| | | | Content | Concentration |
| Young | | | | |
| Estrus | 9 | 14.3 \pm 0.77 | 1973 \pm 410 | 137.6 \pm 28 |
| Diestrus | 6 | 13.5 \pm 0.89 | 1710 \pm 349 | 126.5 \pm 25 |
| Middle-aged | | | | |
| Diestrus | 6 | 14.1 \pm 0.95 | 2323 \pm 386 | 164.8 \pm 27 |
| Constant | 7 | 17.8 \pm 1.14* | 3394 \pm 840† | 190.6 \pm 47 |
| Estrus | | | | |

*Pituitaries significantly ($p < 0.05$) heavier than all other groups.

†Pituitaries serotonin content significantly ($p < 0.05$) greater than two young groups.

^aData from Walker and Cooper (38).

from abnormal exposure of females to estrogen (30,31). In addition to these pituitary effects, estrogen alters CNS monoamine metabolism as described above (32). This effect is expressed ultimately upon the pituitary in which dopamine inhibition of lactotrope function is reduced, while their stimulation by serotonin is increased.

Collectively, these findings suggest that under conditions of monoamine imbalance during aging, abnormal secretion of ovarian estrogen promotes abnormal prolactin secretion leading ultimately to development of pituitary and mammary adenomas. Under such precarious conditions, it would appear that drugs with the potential to further destabilize the reproductive axis should be more toxic in aged than in young individuals.

In an attempt to evaluate this hypothesis, a study was performed to determine if serotonergic drugs synergize with hormonal environments of aged rats to promote tumor development in the pituitary. In the first part of the study, pituitary serotonin content was compared in young and middle aged female rats. The data presented in Table 1 show that mean pituitary serotonin content and concentration were highest in aged rats whose reproductive functions were acyclic. Pituitaries from aged rats that were cycling had serotonin levels that were comparable to young animals. These findings suggest that reproductive status and not age *per se* correlates with changes in the pituitary that are potentially pathogenic.

During the next phase of the study, young rats were given estrogen by subcutaneous implants of silastic capsules that released the steroid abnormally, i.e., in a constant, nonphysiologic pattern. As seen in Table 2, pituitary size and serotonin content were elevated ($p < 0.01$ and $p < 0.05$) in these animals in a fashion resembling that seen in aged rats.

Finally, estrogen-treated rats received serotonin neuroleptics to determine if toxicity of the drugs is enhanced by endocrine conditions simulating those that occur during aging. As seen in Table 3, the tumorigenic effect of estrogen on the pituitary was enhanced in rats receiving concomitant treatment with serotonin recep-

Table 2. Effects of estrogen on pituitary weight and 5-HT content.^a

| Group | N | Weight, g | Pituitary 5-HT, pg \pm SEM | |
|-----------------------------|---|------------------|------------------------------|----------------|
| | | | Content | Concentration |
| Young females | | | | |
| OVX | 5 | 13.6 \pm 0.54 | 1983 \pm 359 | 145.8 \pm 26 |
| OVX + estrogen (5 mmole) | 6 | 36.2 \pm 1.55† | 3120 \pm 415* | 85.1 \pm 11* |
| OVX + estrogen (2 mmole) | 6 | 29.2 \pm 1.06† | 2780 \pm 510 | 95.2 \pm 17 |
| Middle-aged females | | | | |
| Constant estrus | 7 | 17.8 \pm 1.14 | 3394 \pm 840 | 190.6 \pm 47 |
| OVX (previously CE) | 6 | 15.2 \pm 0.95 | 2619 \pm 610 | 172.3 \pm 40 |
| Young males | | | | |
| Castrated | 6 | 14.4 \pm 0.61 | 1629 \pm 439 | 113.1 \pm 30 |
| Castrated + estrogen (5 mm) | 6 | 27.3 \pm 1.01† | 2754 \pm 480* | 100.6 \pm 18 |

*Significantly different ($p < 0.05$) than gonadectomized control.

† $p < 0.01$.

^aData from Walker and Cooper (38).

tor agonists. Rats receiving combined treatment with estrogen and zimelidine had heavier pituitaries ($p < 0.05$) containing more macroadenomas than did rats treated with estrogen alone. None of the serotonin neuroleptics altered pituitary weight or the frequency of adenomas when they were given without estrogen (Table 3).

In contrast to these effects of serotonin receptor agonists, drugs that antagonize age changes have beneficial effects on health. For example, catecholamine precursors and receptor agonists reduce the incidence of neoplastic and nonneoplastic disease during aging (33), restore menstrual bleeding in postreproductive females (34) and stimulate reproductive cycles in aged animals (33,35).

Table 3. Effects of 5-HT receptor agonists and estrogen on pituitary hypertrophy and tumorigenesis in 4-month-old, ovariectomized rats.^a

| Treatment | N | Pituitary weight, (mg-% \pm SEM) | Number of rats with macroadenomas |
|-------------------------|---|------------------------------------|-----------------------------------|
| | | | |
| OVX ^b | 4 | 12.0 \pm 0.82† | 0 |
| Estrogen | 5 | 36.6 \pm 3.95 | 0 |
| Estrogen + PCPA | 6 | 32.2 \pm 2.87 | 0 |
| Estrogen + 5-HTP | 7 | 38.4 \pm 3.54 | 0 |
| Estrogen + zimelidine | 7 | 78.6 \pm 18.00* | 3 |
| Estrogen + quipazine | 5 | 53.2 \pm 13.60 | 1 |
| Zimelidine ^b | 4 | 14.2 \pm 0.71* | 0 |
| Quipazine ^b | 4 | 12.6 \pm 0.69† | 0 |

^aData from Walker and Cooper (38).

^bAnimals had empty Silastic capsules implanted subcutaneously.

†The pituitary weight of these three groups was significantly smaller ($p < 0.05$) than those groups receiving estrogen implants.

*Significantly larger ($p < 0.05$) than all other groups except estrogen + quipazine.

Discussion and Conclusion

The major goal of this study was to highlight peculiarities of reproductive function in aging females that provide an environment in which potential toxicity of certain drugs can be expressed. In this regard, it is not the obvious deficit in ovarian activity seen in late postmenopausal women that is of concern. Instead, the erosion of integrated function within the reproductive neuroendocrine axis that begins and progresses throughout middle-age creates an endocrine milieu with pathogenic potential that is amplified by certain drugs. Although this progressive dysregulation makes most, if not all, organs of the body more vulnerable to disease, the present study focused upon the reproductive axis *per se*, considering the effects of abnormal estrogen and prolactin secretion in conjunction with monoamine neuroleptics upon development of pituitary adenomas. Specifically, clinical and experimental evidence was provided to show that ovarian secretion of estrogen does not simply decline with advancing age. Instead, irregular reproductive cycles beginning in middle age are accompanied by abnormal secretion of the steroid. There is not a precise pattern of estrogen release during this period of instability, but, in general, secretion of the hormone is prolonged as the pattern of phasic or intermittent release seen in youth is lost. The cause(s) of perturbations in ovarian function are unknown, but in rodent models they seem to result from central neurotransmitter changes that alter the pattern of gonadotropin release from the pituitary.

In any event, it is proposed that the protracted release of estrogen resulting from such central monoaminergic perturbations exacerbates the primary lesion by altering the metabolism of serotonin and catecholamines. This effect also initiates catastrophic feedback processes involving prolactin that lead to irreversible degeneration of the gonad. Under these conditions, drugs with estrogenic or serotonergic action are expected to further promote dysregulation and thus toxicity, which would not occur in the youthful, functionally integrated reproductive axis. Specifically, it was shown that the serotonin receptor agonists quipazine and zimelidine enhanced the hypertrophic effect of estrogen upon the pituitary and increased the incidence of tumors in that tissue.

Differential toxicity of these drugs is dependent upon endocrine background, as demonstrated by the fact that neither of the neuroleptics were tumorigenic in the absence of estrogen. Thus, drug-induced pituitary tumors in the rodent model seem to be secondary to changes in neuroendocrine events that make the animal more vulnerable to negative aspects of the drug action. The findings of this study that consider serotonergic neuroleptics and pituitary adenomas may have clinical relevance, since drugs such as bromocryptine that are currently used to regress lactotroph adenomas in human (36) possess weak antiserotonergic activity (37) in addition to their dopamine agonistic effects. Perhaps, in the future, the use of drugs that are strong serotonin

receptor antagonists in addition to being dopamine agonists will prove beneficial to the management of patients with certain types of pituitary tumors.

In conclusion, the purpose of this report is to suggest that the scope of studies in reproductive toxicology should be expanded to consider the effects of drugs in individuals beyond their reproductive years, as well as in those that are reproductively competent. These two focuses are different in that traditional concern has been 1800for maintaining fertility and preventing congenital defects. However, drug toxicity related to reproductive function in aging individuals is another equally valid issue, and with the current expansion of the geriatric population, it is one that undoubtedly deserves attention.

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